USE OF DIFFERENTIAL GENE EXPRESSION ANALYSIS TO STUDY SOMACLONAL VARIATION IN OIL PALM

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Introduction

Micropropagation of oil palm by somatic embryogenesis provides a useful means of exploiting elite genotypes of this economically important species. Clonal progeny obtained by this approach have however been found to contain a variable percentage (averaging 5-10%) of palms bearing the *mantled* floral abnormality [1] (Fig. 1). The latter involves an overall feminisation of both male and female flowers: in the latter, the staminodes (vestigial stamens) develop as pseudocarpel structures which may develop after fertilisation to give *mantled* fruit. The most severely affected palms produce sterile flowers; thus the oil production of the plantation is reduced. Several key features of the *mantled* abnormality reveal that it is epigenetic in nature. Firstly, it has been observed that reversion to a normal floral phenotype may occur over a period of years following the onset of flowering. Secondly, although the *mantled* abnormality is strongly transmitted through tissue culture, only a weak non-Mendelian transmission of the *mantled* character is observed via seeds. Thirdly, the fact that the abnormal phenotype is unlikely to have resulted from a classical genetic mutation is borne out by ploidy, RAPD and AFLP studies previously carried out in our lab [5, 4].

Experimental Approaches

Given that the *mantled* character is epigenetic in nature, we have used an experimental approach based on differential display analysis [3] as a means of identifying genes which are differentially expressed in a *mantled*-related fashion (Fig. 2).

Results

Figure 3 summarises the data obtained thus far for potential markers of the *mantled* abnormality identified by differential display RT-PCR. Starting from a total of 46 differential bands observed on differential display gels, 13 markers have been validated by Northern hybridisation, of which 6 correspond to genes which are more highly expressed in normal tissues and 7 to genes which are more highly expressed in abnormal tissues. Figure 4 shows a Northern hybridisation performed to determine the tissue specificity of one of the markers producing a stronger signal in abnormal tissues. In this case it can be seen that differential expression occurs not only in shoot apex segments, but also in callus, in which the differential expression pattern is even more marked.

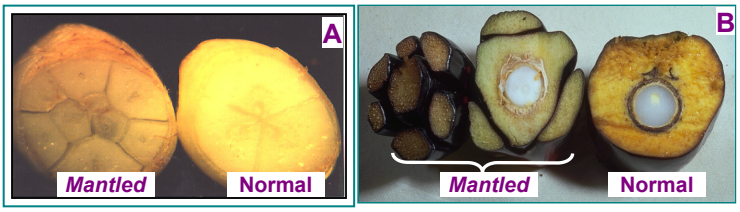

Fig. 1. Morphology of normal and *mantled* oil palm flowers (A) and fruits (B).

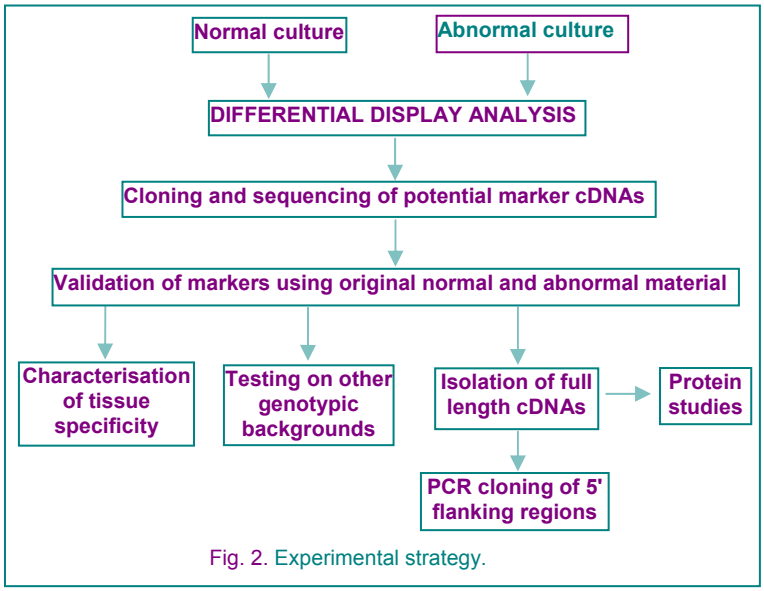

Fig. 2. Experimental strategy.

No of potential markers identified by differential display	46
No of individual cloned cDNA fragments obtained	58
No of cDNAs producing differential signals in Northern retesting	13
No of cDNAs producing stronger Northern signal in normal tissue	6
No of cDNAs producing stronger Northern signal in abnormal tissue	7

Fig. 3. Summary of data obtained to date on differential display markers.

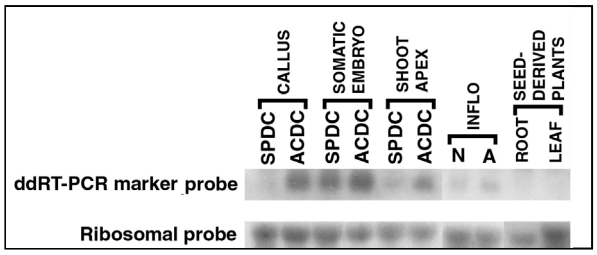

Fig. 4: Northern hybridisation analysis of the tissue specificity of a putative marker of abnormal tissues. Abbreviations: SPDC, normal seed palm-derived culture; NCDC, ACDC, abnormal (*mantled*) clone-derived culture; INFLO, normal or mantled inflorescence (N or A).

Conclusions

The availability of cDNAs corresponding to genes which are differentially regulated with respect to the *mantled* abnormality will allow us to study the molecular mechanisms by which tissue culture stress eventually leads to the reprogramming of gene expression in the oil palm flower. Recent studies have shown that the *mantled* abormality is associated with genomic DNA hypomethylation [2] and it will be interesting to study this phenomenon for specific genes and their promoter sequences. From a practical point of view, it is hoped that the availability of *mantled* markers will allow the refinement of tissue culture regeneration protocols and/or the establishment of a clonal conformity test. This in turn will allow the use of somatic embryogenesis as a large scale micropropagation method for oil palm.

References

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